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Introduction

There is an abundant amount of evidence suggesting that catecholamines administered centrally can regulate adrenocorticotropin (ACTH) and vasopressin (AVP) release (2,4,33,39). Because there is a heavy catecholaminergic projection from the brainstem innervating the paraventricular nucleus (PVN) of the hypothalamus (36,38), the nuclear region that regulates AVP and ACTH release from the pituitary (16,18,20), it has been suggested that catecholaminergic regulation of ACTH and AVP may occur directly at this site (2,4). Also, these catecholaminergic cell groups are located in regions involved in the regulation of baroreflexes and the regulation of arterial blood pressure and heart rate during hemorrhage (9,10,17,31,36,38,40). Recently, type II glucocorticoid receptors have been found on catecholaminergic cell groups in the brainstem (22). Coupled with the findings that ACTH and AVP immunostaining (7,8,27) and mRNA levels (6,19) are altered after adrenalectomy, these data suggest that 1) the responses of plasma ACTH and AVP and the recovery of arterial blood pressure and heart rate after hemorrhage and 2) the baroreflex, may be influenced by glucocorticoids which may work though these catecholaminergic cell groups.

The goal of this project was 1) to determine if catecholamines (and other putative neurotransmitters) lead to systemic release of ACTH and AVP and regulate arterial blood pressure and heart rate, 2) to determine if the loss and replacement of glucocorticoids effects baroreflex function, 3) and to determine if the recovery of arterial pressure and heart rate and the responses of plasma ACTH, AVP, oxytocin and norepinephrine to hemorrhage are effected by the loss of glucocorticoids. Also, since recent evidence in our laboratory strongly suggest that the nutritional state of the animal effects the ability of an adrenal insufficient animal to recover arterial pressure after hemorrhage, and that glucocorticoids are critical for the mobilization of body fuels during stress, possibly through brainstem

catecholamine centers (25) and the PVN (28), we have also charactorized the responses of the above variables in fed and fasted states.

Material and Methods

Microinjections into the PVN: Male Sprague-Dawley rats (250-350) were anesthetized with pentobarbital sodium (45mg/kg,ip) and cannulae were placed in the femoral artery and vein (PE-50) for measurement of arterial pressure, heart rate and for blood withdrawal. The rats were placed in a stereotaxic device, the skin and muscle of the cranium was retracted, and a burr hole was drilled (-1.4 to -1.6mm from bregma, 0.4mm lateral from midline and 7.0-7.4mm deep). Each rat was allowed 1 hour to stabilize before any further manipulation. Arterial blood pressure and heart rate were monitored continuously throughout each experiment. A glass micropipette (approx. 50μm OD), filled with freshly prepared norepinephrine, phenylephrine or clonidine (10^{-9} to 10^{-2}M) in artificial CSF, or artificial CSF, or L-glutamate (0.5M) or acetate (0.5M) was lowered into the PVN. Ten minutes later, 50nl was injected over two minutes. Injections were unilateral. Blood samples (1ml) were taken before and 5, 10 and 20 minutes after the completion of the injection. Some animals were pretreated with atropine (2mg/kg), propranolol (1.5mg/kg) or pentolinium (20mg/kg) 10minutes before measurements were taken.

Baroreflex studies: Male Sprague-Dawley rats weighing 275-350 gram were chronically cannulated as described previously (14,17,34). Briefly, the rats were anesthetized with pentobarbital sodium (45mg/kg, ip), and femoral vein (PE-50) and artery (Dural Plastics) cannulaes were placed using sterile procedures. The cannulaes were tunneled under the skin of the back and through a spring the was attached to the back of the neck and the top of the cage. All incisions were filled with xylocaine jelly and polysporin (Burroughs-Wellcome) to desensitize the surgical area and to prevent infection. The rats recovered and were caged singly in a room (controlled temperature and humidity) with a 12-h on/off light cycle. All rats had access to food and water ad libitum. After 3 days, the rats were either bilaterally adrenalectomized or sham adrenalectomized under ether anesthesia. Corticosterone replacement was effected by placing fused pellets of 20,40, or 80% corticosterone-cholesterol (approx 100mg) or wax pellets under the skin of the back as described by Akana et al. (1). The rats recovered and were all given 0.5% saline to drink and food ad libitum. Six days later, while fully conscious, the arterial cannulaes were connected to a Statham pressure transducer and polygraph for measurement of arterial blood pressure and heart rate. All cannulaes were manipulated outside the cage so as not to disturb the animals. Varying doses of the alpha-1 agonist phenylephrine and nitroglycerin were injected intravenously while monitoring arterial blood pressure and heart rate.

The responses of heart rate to varying levels of arterial pressure were compared by using a step-wise polynomial regression while testing the highest coefficient for statistical significance. Coefficients were then compared by t-test.

Responses to Hemorrhage: Male Sprague-Dawley rats were chronically cannulated as described above. Three days later, the rats were either bilaterally adrenalectomized or sham adrenalectomized under ether anesthesia and all rats were given 0.5% saline to drink. All rats had access

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to food and fluids ad libitum. Five days later, all rats recieved a 15ml/kg *5minute hemorrhage through the arterial cannulae. 2.5ml of blood were taken 20, 60, 120 and 300 minutes after hemorrhage for determination of plasma corticosterone, AVP oxytocin, renin and norepinephrine. Each blood sample was centrifuged, and the required volume of plasma was removed and put on ice. An equal amount of saline was added to the red cells and whole blood (from hemorrhage) not used for determination of plasma constituents. This saline-red cell mixture was returned to the rat (via the venous cannulae) as arterial samples were taken. Arterial blood pressure and heart rate were monitored continuously when samples were not taken.

Responses to Hemorrhage, Fed and Fasted: Male Sprague-Dawley rats weighing between 280-350 grams were chronically cannulated and adrenalectomized as described above. Four days after adrenal surgery, food, but not saline, was taken away from half of the rats. On the morning of the 5th day after adrenal surgery, hemorrhage (15 ml/kg *5min) was performed though the arterial cannulae. Further 2.5ml blood samples were withdrawn at 20, 60, 90, 120 and 300 minutes for measurement of plasma ACTH, corticosterone, glucose, lactate, B-hydroxybutyrate, alanine, aspartate transaminase, alanine transaminase and alkaline phosphotase. 0.2ml blood samples were additionally taken at 10, 45 and 180 minutes for measurement of plasma glucose, lactate, B-hydroxybutyrate and alanine. The time 0 measurement of these plasma constituents was derived from the hemorrhaged blood. The red blood cells derived from the hemorrhage and sampling were mixed with an equivalent volume of saline and reinfused through the venous cannulae as blood samples were taken from the arterial cannulae. Some adrenalectomized rats had an infusion of glucose started at 1hr into hemorrhage (60mg/hr). Some adrenalectomized rats had an infusion of corticosterone (70μg/hr) at time of hemorrhage. Some adrenalectomized rats had a 30% (corticosterone/cholesterol) pellet placed under the skin at the time of adrenalectomy.

Arterial blood pressure and heart rate were measured before and after hemorrhage via the arterial cannulae as described above. Plasma glucose concentration was measured by the glucose-oxidase technique (Beckman Glucose Analyzer II). Plasma lactate, B-hydroxybutyrate and alanine concentrations were determined by the Ezymatic Methods of Bergmeyer (5). Plasma aspartate transaminase, alanine transaminase and alkaline phosphotase were measured by kit (Sigma). Plasma ACTH and corticosterone were measured by RIA as described below.

Data were analyzed for statistical significance using a 1-way ANOVA for analysis of response over time and a 2-way ANOVA corrected for repeated measures over time to compare the responses between groups. Newman-Keuls multiple range test was used to compare means after ANOVA. Significance indicated at $P < 0.05$.

Hormone Assays: Plasma ACTH was measured by radioimmunoassay (RIA) from glass-extracted plasma as described previously (13). The lowest detectable concentration was 10 pg/ml: intra- and interassay variations were 7 and 9.5% respectively. Plasma corticosterone was determined from heat denatured samples (44) with inter and intraassay coefficients of variation of 8 and 9% respectively. The minimal detectable level in a 10ul sample was 0.1μg/dl. Vasopressin was assayed by RIA from bentonite-extracted plasma (41). The lowest detectable concentration was

0.3pg/ml;intra and interassay variations were 9 and 12% respectively. Oxytocin was measured by RIA in acetone extracts from plasma (26). The inter- and intraassay coefficients of variation were 3.2 and 6.8% respectively, and the lowest detectable level was 0.8pg/ml. Plasma renin concentration was determined by adding 0.1ml sample plasma to 0.9ml nephrectomized rat plasma for generation of angiotensin I in vitro at pH 6.5 for 2 hours. Angiotensin I was determined by RIA (37). The inter- and intraassay coefficients of variation were 5.2 and 11.3% respectively and the lowest detectable level was 1ng AI/ml *2h. Plasma norepinephrine was determined by radioenzymatic assay (32) or by HPLC with electrochemical detection.

Results

Microinjections into the PVN: Because brainstem catecholaminergic cell groups have been implicated in the regulation ACTH and AVP release at the level of the PVN, we have been microinjecting various catecholamines into the PVN and measureing plasma ACTH and AVP. We have found that microinjections of norepinephrine led to a dose- dependent elevation in plasma ACTH (Figures 1 and 2) however, microinjections of norepinephrine had no effect on heart rate or arterial blood pressure. Also, microinjection of 10^{-4}M norepinephrine into the PVN lead to a rise in plasma AVP. Control injections of vehicle and injections of norepinephrine outside the nucleus had no effect (Figure 2)

Microinjections of L-glutamate into the paraventricular nucleus led to the rise in plasma adrenocorticotropin, vasopressin and oxytocin (Figure 3). Also, activation of these neurons led to a profound bradycardia with little change in arterial blood pressure (Figures 3 and 4). This response was both dose and volume dependent (Figure 5). This finding was confirmed by focal electric stimulation of the paraventricular nucleus and surrounding nuclear structures (Figure 6). Electric stimulation of the paraventricular nucleus led to a decrease in heart rate that was dependent on current amplitude and frequency (Figure 7). The greatest drop in heart rate and blood pressure occuring at 50-100μA and at 50hz. Also, this effect is most likely mediated through the parasympathetic nervous system since atropine abolishes most of the bradycardia (Figure 8). This effect could only be produced by glutamate injection into the paraventricular nucleus and not in any of the surrounding nuclei in the hypothalamus and thalamus (Figure 9). From this data we propose that L-glutamate activates neurons in the paraventricular nucleus that project to and stimulate 1) the preganglionic parasympathetic neurons in the nucleus ambiquus and dorsal motor nucleus of the vagus and inhibit 2) the preganglionic sympathetic neurons in the intermediolateral column of the spinal cord (Figure 10).

Baroreflex studies: Brainstem catecholaminergic cell groups have also been implicated in the regulation of baroreflex function and these neurons have been shown to contain the type II glucocorticoid receptor. To determine if loss and replacement of glucocorticoids effects baroreflex function, we recorded the changes in heart rate while increasing and decreasing MABP with varying doses of phenylephrine and nitroglycerine in conscious rats that were adrenalectomized and given corticosterone (in pellet form, 0, 20, 40 and 80% mixed with cholesterol) at the time of adrenal surgery. Baroreflex curves were determined and it was found that the curves were significantly different between ADRX and Sham rats (Figure

12). We also found that the best replacement dose that corrected the reflex curve was the ADRX + 80% pellet group (corticosterone levels of 5.1 $\pm$ 0.4 μg/dl) where the 40% and 20% replacement groups (2.1 $\pm$ 0.5 and 1.0 $\pm$ 0.2 μg/dl, respectively) had baroreflex curves that were not different from ADRX + 0% (Figure 12). The levels of corticosterone (80% group) that were necessary to restore baro-function were much higher then those levels need to restore body weight, thymus weight and plasma CBG levels to normal (40% pellet, ref.14). Upon further analysis of the baroreflex curves, it was found that the slopes of the regressions though the phenylephrine side of the curves (increase in MABP) were not different between groups however, the slopes of the nitroglycerine part of the curves were different between the Sham and ADRX + 0% groups; the ADRX + 80% pellet group had a slope not different from sham (14). This suggests that the baroreflex may be regulated by two systems. One that regulates heart rate during hypertension and one that regulates heart rate during hypotension. It is the latter system that appears to be affected by glucocorticoids.

Responses to Hemorrhage: Since brainstem catecholaminergic groups contain glucocorticoid receptor and, are located in areas involved in the regulation of arterial blood pressure during hypovolemia, we have studied the recovery of arterial blood pressure and have charactorized the responses of various hormones and blood constituents to hemorrhage in the adrenalectomized rat. These have been difficult studies to perform in the past because adrenally insufficient animals have a very high mortality rate. However, we have developed a conscious adrenalectomized model that is healthy and viable.

We have now shown that the recovery of mean arterial blood pressure (MABP) to 15ml/kg *5min hemorrhage in the adrenalectomized (ADRX) rat is almost identical to that in sham rats (Figure 13). This was a surprising in lue of a large literature demonstrating that ADRX rats, cats, dogs, and adrenally insufficient humans are very fragile and do not survive this type of stress. Further studies in this lab have revealed that the recovery of MABP in ADRX rats is probably due to the potentiated recovery of heart rate (Figure 13), and the potentiated responses of vasoactive hormones: vasopressin, oxytocin, renin and norepinephrine (Figure 14). These data suggest that some component, possibly a neural one, has changed the gain of the system.

Responses to Hemorrhage, Fed and Fasted: Glucocorticoids have long been implicated in the regulation of metabolism though various central structures, possibly through brainstem catecholaminergic neurons . These studies were performed to charactorize the responses of the cardiovascular and hormonal systems to hemorrhage in fed and fasted conscious rats that have been given food ad libitum or have had it removed prior to hemorrhage.

As reported above, all fed, adrenalectomized rats lived though the hemorrhage and subsequent 24 hours. By contrast, all fasted adrenalectomized rats died within 24 hours of the hemorrhage, most of them between 2.5 and 3.5 hours after the hemorrhage volume was removed. The responses in MABP and heart rate in both fed and fasted adrenalectomized and sham-adrenalectomized rats are shown in Figure 15. Fed, adrenalectomized rats restored and maintained MABP well compared to shams. By contrast, fasted adrenalectomized rats did not sustain MABP, demonstrating a slow decline until death occurred even though the initial

return of MABP resembled the return of MABP in the fasted sham controls. Initial heart rate was elevated in both groups of adrenalectomized rats compared to the sham controls. After the reflex bradycardia occasioned by the hemorrhage, heart rate continued to be elevated compared to shams for several hours.

The responses of plasma ACTH and corticosterone to hemorrhage are shown in Figure 16. All groups demonstrated an elevation of plasma ACTH to hemorrhage from basal levels. Initial ACTH concentrations were elevated in the adrenalectomized rats compared to the shams (Fig. 16, top), and there was no corticosterone response to the hemorrhage in these groups (Fig. 16, bottom). By contrast, the magnitudes of the ACTH responses (change) to hemorrhage were similar in fed and fasted, adrenalectomized and sham adrenalectomized rats suggesting that the neural elements that regulate ACTH release have changed their operating set point and not the gain of the system. The ACTH response persisted for approximately 2 hours and hormone concentrations had returned to initial levels by 5 hours in the rats that survived.

Plasma concentrations of energy substrates are shown in Figure 17. In fed, sham adrenalectomized controls, plasma glucose concentrations did not change after hemorrhage. By contrast, in fed, adrenalectomized rats glucose concentrations fell between 1 and 2 hours and remained significantly lower than the fed sham group. Initial plasma glucose concentrations were decreased in both groups of fasted rats. Fasted sham adrenalectomized rats increased their plasma glucose levels with time after hemorrhage so that by 5 hours the levels were elevated above initial values. Plasma glucose levels fell in fasted adrenalectomized rats, and from 90 minutes until death there was a progressive and marked decline in these levels.

Plasma lactate concentration demonstrated a biphasic increase to hemorrhage in all groups (Figure 17, 2nd panel). However, the fasted adrenalectomized group showed an exaggerated response as compared to the fasted sham group. Plasma B-hydroxybutyrate concentrations did not change with time after hemorrhage in the fed groups. By contrast, fasting resulted in elevated initial values of this fatty acid, and hemorrhage occasioned an increase in the sham adrenalectomized, but not the adrenalectomized rats (Figure 17, third panel, left and right). Plasma concentrations of alanine were only marginally affected in these experiments with minor elevations occurring in the fasted adrenalectomized rats at 90 and 180 minutes (Figure 17, bottom panel, left and right).

The response of plasma vasopressin, renin and norepinephrine to hemorrhage was significantly potentiated in the fasted adrenalectomized groups as compared to the fasted sham group (Figure 18). Infusion of glucose did not prevent death (Figure 19) and the hormonal responses were similar to the vehicle infusion group (ADRX). Infusion of corticosterone during hemorrhage (acute B group, Figure 19) prevented death in approximately half of the group and had hormonal responses that were similar to the vehicle infusion group (ADRX). However, chronic infusion of corticosterone decreased the potentiated hormonal responses as compared to the vehicle infusion group and prevented death in the fasted adrenalectomized rat (Figure 19).

Plasma apartate transaminase was significantly elevated after hemorrhage in the fasted adrenalectomized group as compared to the fasted sham group (Figure 20) suggesting that liver failure is occuring in the

fasted adrenalectomized rat. There was no difference in plasma alanine transaminase and alkaline phosphotase between fasted adrenalectomy and sham groups after hemorrhage.

Discussion

Microinjections into the PVN: There is both anatomic and physiologic evidence for the role of central catecholaminergic regulation of basal and stress induced release ACTH and AVP. Catecholaminergic pathways between the nucleus of the NTS and PVN have previously been described and include 1) noradrenergic and adrenergic cell bodies in the caudal extent of the NTS (A2,C2 cell groups) and 2) the A1,C1, A5 and A6 cell groups (9,10,36,38). All these groups receive hemodynamic information (changes in blood pressure and blood volume) and project to the PVN. Thus, the evidence for catecholamines being the anatomical stubstrate for regulation of ACTH and AVP release is strong.

Physiologic evidence for catecholaminergic regulation of the adrenocortical system is also strong. Destruction of catecholamine cell groups by central injection of 6-hydroxydopamine (6-OHDA) results in disruption of both basal and stress-induced activity in the adrenocortical system (see Review in 12). Moreover, catecholamines injected into the brain will elevate plasma ACTH (33,39) and AVP (4). However, it is not clear what receptor type is involved nor if the PVN is the site of action of the catecholamines.

From the data presented here (Figure 1), it appears that the alpha agonists, phenylephrine and clonidine, are not as potent as norepinephrine for stimulation of ACTH release when injected in the PVN. However, these experiments are incomplete and lack injections of beta adrenergic agonists and of the alpha agonists at higher doses.

The Paraventricular nucleus has been described as containing neurons that regulate both pituitary AND autonomic function (36,38). This laboratory (Figures 1 and 2) and others (4,33,39) have shown that catecholamines AND L-glutamate stimulate ACTH and AVP release when microinjected into the paraventricular nucleus. However, microinjections of norepinephrine at any dose did not lead to changes in arterial blood pressure or heart rate where microinjections of L-glutamate did. Because L-glutamate is a nonspecific excitatory neurotransmitter that (probably) stimulates all neurons in the PVN, our data stongly suggests that brainstem ascending norepinephrine system to the PVN can stimulate neurons that control pituitary output but do not influence PVN neurons that regulate autonomics.

Baroreflex studies: Glucocorticoid receptors have been found in cell located in medullary structures known to mediate baroreceptor signals and regulate sympathetic output (22). These data demonstrate that complete loss of corticosterone will alter barofunction (Figure 12) that only the high 80% pellet would normalize. Since the normal circadian rhythm for corticosterone in rats ranges from 0.5 ± 0.1ug/dl in the morning to 9.0 ± 2.3ug/dl in the evening (as measured in these rats), the evidence in Figure 12 suggest that it is the evening rise in corticosterone that allows for normal baroreceptor function. Also, it appears that adrenalectomized rats have an abnormal nitroglycerin-induced baroreceptor curve while the phenylephrine-induced curve was normal (14). This suggests that

corticosterone effects only one side of the baroreceptor curve; regulation of heart rate as pressure falls.

Responses to Hemorrhage: These results demonstrate that recovery of MABP in the adrenalectomized (ADRX) conscious rat is similar to that in the Sham rat (Figure 13); however, responses in heart rate, vasopressin, oxytocin, renin and norepinephrine were all potentiated. The restitution of blood volume in the ADRX rat, although lagging behind that of the sham group, was shown to be over 100% by 5 hours (15). This lag in the restitution of blood volume in the ADRX group may be due to the fall in plasma osmolality in the ADRX group instead of a rise (demonstrated by the Sham group) and not due to a deficit in plasma protein restitution since its recovery was not different from that measured in the Sham group (15).

Under normal conditions, the recovery of MABP after hemorrhage involves brain stem centers that coordinated actions of the autonomic nervous system and the pituitary for regulation of vasoconstriction and fluid homeostasis. These results show that conscious ADRX rats can regulate their arterial blood pressure at levels similar to those in sham rats, even though there is a lag in the restitution of blood volume, by elevating heart rate (thereby elevation cardiac output) and vasoactive hormones (Figures 13 and 14).

Responses to Hemorrhage, Fed and Fasted: Fed and fasted sham-adrenalectomized rats restored arterial blood pressure to near normal values within 60 minutes of hemorrhage, and sustained this over the 5 hour period of study (Figure 15). Although plasma glucose levels were fairly constant in the fed control rats, glucose production appeared to have been increased in the fasted rats after 2 hour. Similarly, plasma B-hydroxybutyrate concentrations remained fairly stable in the fed control rats whereas plasma concentrations of this energy substrate tended to be an increase in the fasted rats during the first 2 hour after hemorrhage (Figure 17). Thus, the fasted control animals appeared to mobilize substrate after hemorrhage, first in the form of fatty acids and subsequently in the form of glucose. The timing of such responses is compatible with the known actions of elevated levels of vasopressin, norepinephrine and glucocorticoids secreted in response to the hemorrhage (15,18,29). The fact that responses of similar magnitude in circulating energy substrate levels did not occur in the fed controls suggests that the hormonal responses were counteracted by other means.

The major differences in responses between fed and fasted adrenalectomized rats which we measured, were those that began to occur 60 minute after hemorrhage. At about this time, both MABP and plasma glucose levels began to decline slowly in the fasted, adrenalectomized rats. The decline in these variables was sustained for the next few hours, until death usually occurred. Plasma B-hydroxybutyrate levels did not rise in the fasted adrenalectomized rats as they did in the fasted controls, but instead declined slowly during the post-hemorrhage period. The lowest level of this substrate observed in adrenalectomized rats was equivalent to concentrations in the fed animals. Since muscle preferentially uses fatty acids for energy under conditions of starvation, it may be that the slow fall in this substrate in fasted adrenalectomized rats was also a factor contributing to death.

The finding that there is a strong interaction between feeding state and adrenalectomy on survival after hemorrhage stress, strengthens our

previously articulated hypothesis that a primary phyiological role of the adrenocortical system is to serve as an efferent arm of a larger hypothalamic system involved with caloric intake, storage and mobilization (11). The activity of the adrenocortical system is quantitatively responsive to caloric restriction (23,24,42,43), and death that occurs after hemorrhage in fasted, adrenalectomized rats, but not fasted controls, suggests that the fasting-induced rise in corticosteroids may play a life-saving role in the capacity to withstand stress.

Even though the fall in plasma glucose and ß-hydroxybutyrate suggests that the loss of these body fuels in the fasted adrenal insufficient rat are linked to mortality after hemorrhage, infusion of glucose after hemorrhage did not prevent cardiovascular collapse and death (Figure 19). Nor did acute infusion of corticosterone guard against mortality. However, the chronic presence of low levels of corticosterone protected the fasted adrenalectomized rat from hemorrhage (Figure 19). These data suggest that 1) basal levels of adrenal steroids are nessecary to prime or allow the normal compensatory mechanisms to restore blood pressure and blood volume after hemorrhage and 2) the fasted adrenalectomized rats are probably dying with some degree of liver failure. This last point is made because of 3 facts. In a fasting rat, almost all of the glucose and ß-hydroxybutyrate is made by the liver. Therefore, if glucose and ß-hydroxybutyrate levels in the plasma fall, this suggests that liver function is impaired. Also, plasma corticosterone levels plateaued during infusion in the fasted adrenalectomized rats that survived and steadly increased in those that did not (Figure 19, 3rd row, 4th panel). Since the liver is the main site of corticosterone metbolism, the rise in plasma corticosterone during infusion suggests that it is not being metabolized, suggesting liver failure. And lastly, plasma aspartate transaminase levels are significantly higher after hemorrhage than in the fasted sham group, again suggesting liver failure (Figure 20). Because plasma glucose and ß-hydroxybutyrate fall at the same time that arterial pressure falls, the cause of cardiovascular collapse after hemorrhage in this model may not be due to liver failure, but a consequence of the fall in arterial pressure.

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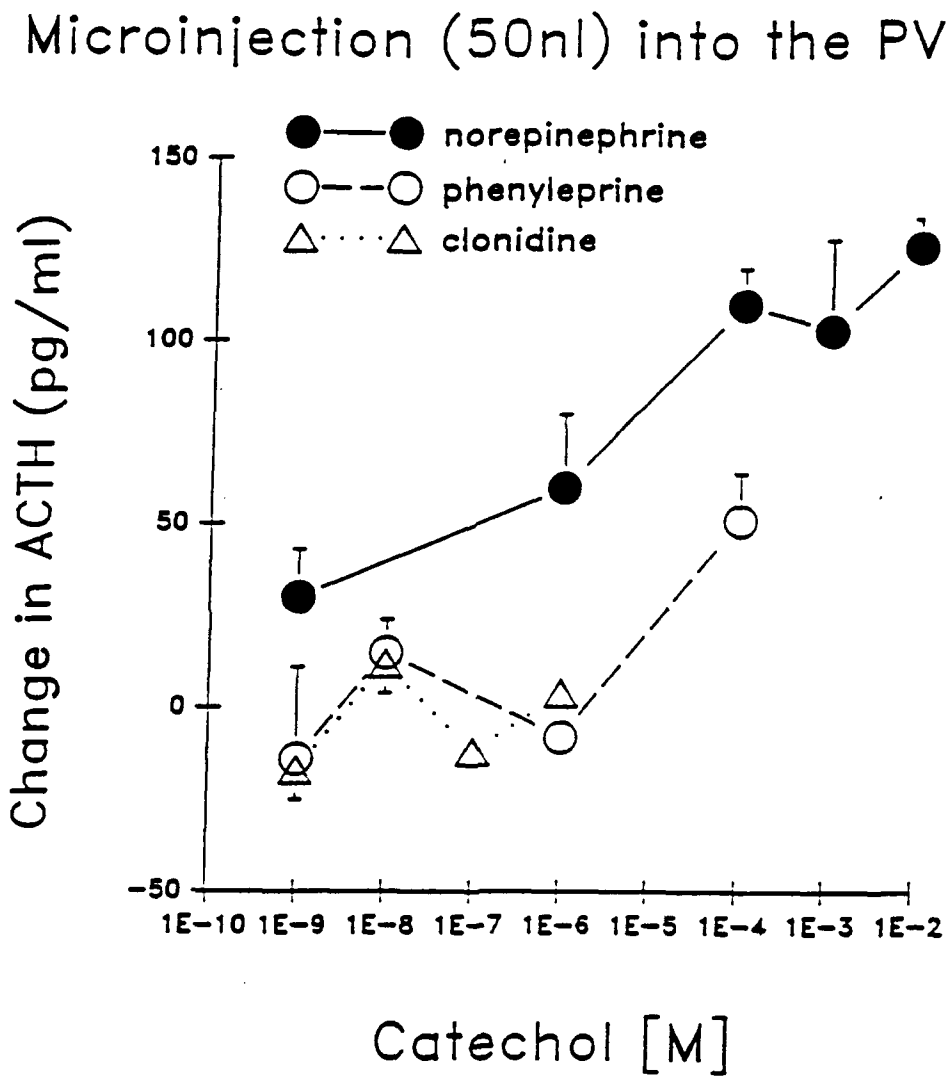

Figure 1. Dose-response curve for plasma ACTH after microinjection (50nl) of varying concentrations of catecholamines into the paraventricular nucleus of the hypothalamus in pentobarbital-anesthetized rats.

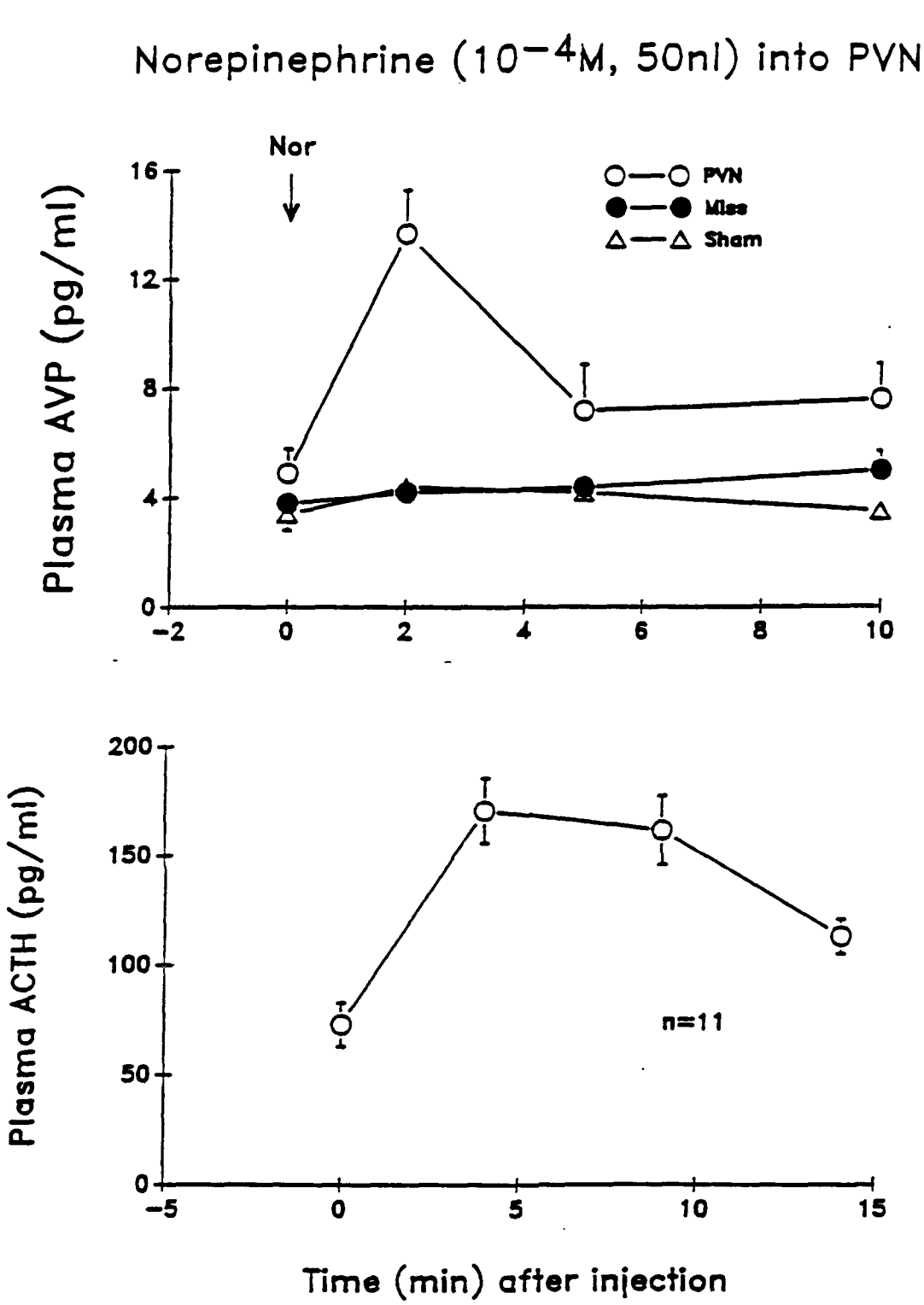

Figure 2. Plasma ACTH and vasopressin (AVP) levels before and after unilateral microinjection of 10^{-4}M norepinephrine into the paraventricular nucleus (PVN) or into structures surrounding the PVN (Miss) or injections of vehicle (Sham).

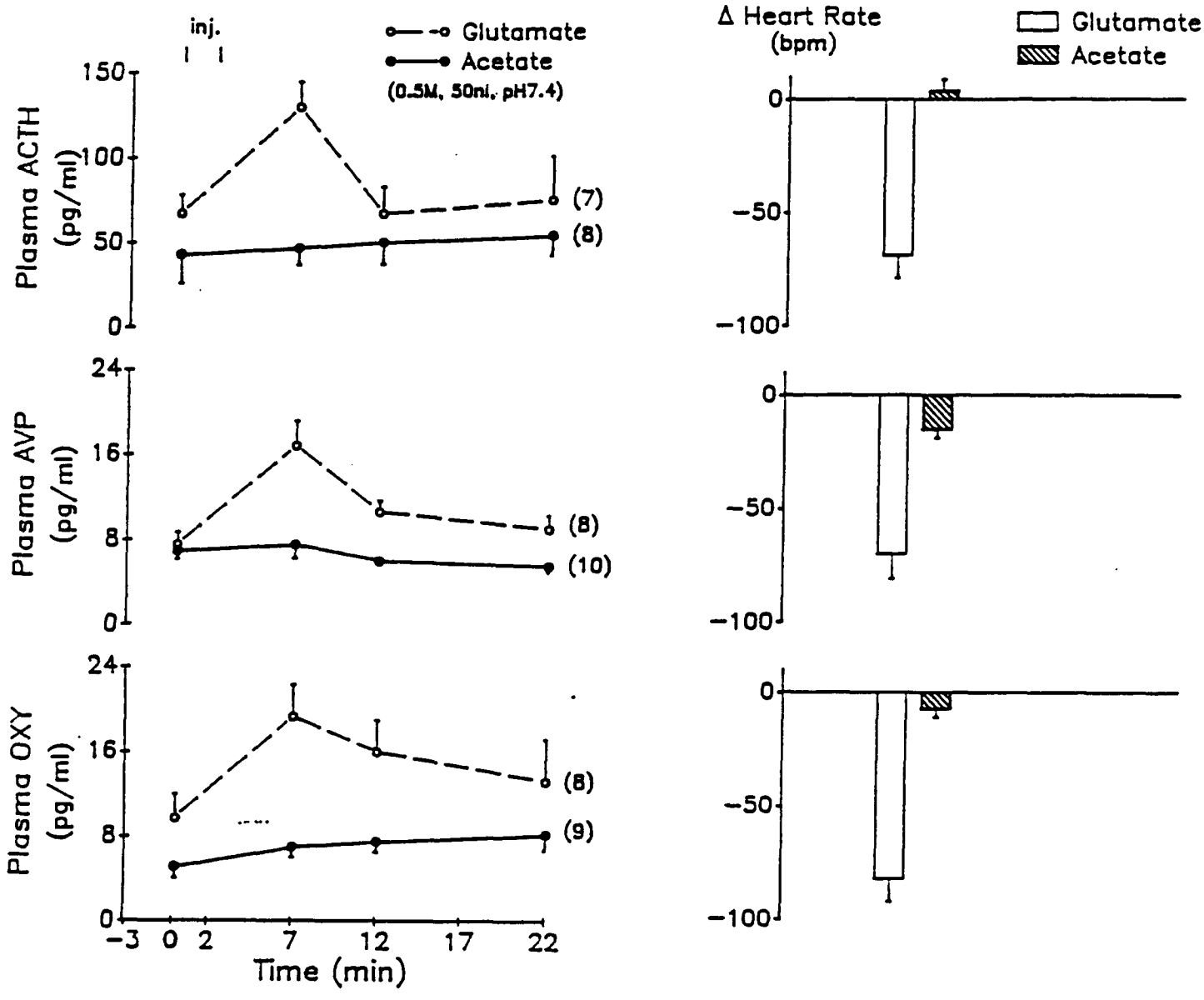

Figure 3. Plasma levels of ACTH, vasopressin and oxytocin in response to activation of cell bodies in the paraventricular nucleus (PVN) with L-glutamate. Right side of Figure shows corresponding heart rate changes. All glutamate-induced responses are significant and different from the acetate-induced responses. Values represent mean $\pm$ SE. inj.- injection period. Numbers next to each graph represent the number of successful injections into the PVN.

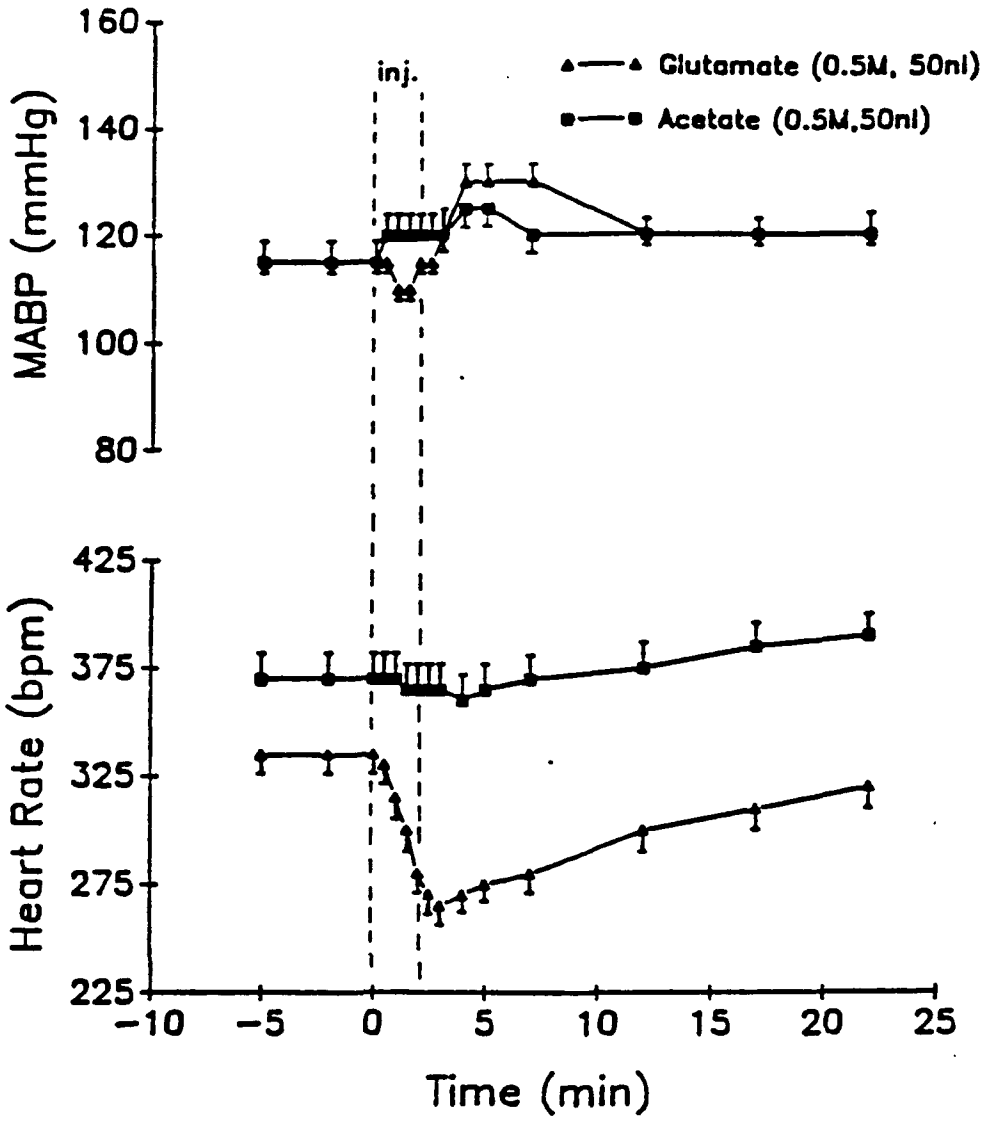

Figure 4 shows the responses of mean arterial blood pressure (MABP) and heart rate to activation of cell bodies in the PVN by a 2 minute unilateral microinjection of 50nl of L-glutamate or acetate. The response of heart rate to microinjection of L-glutamate is significant and different from the response to acetate. Values represent mean $\pm$ SE. Glutamate - n = 39; Acetate - n = 13

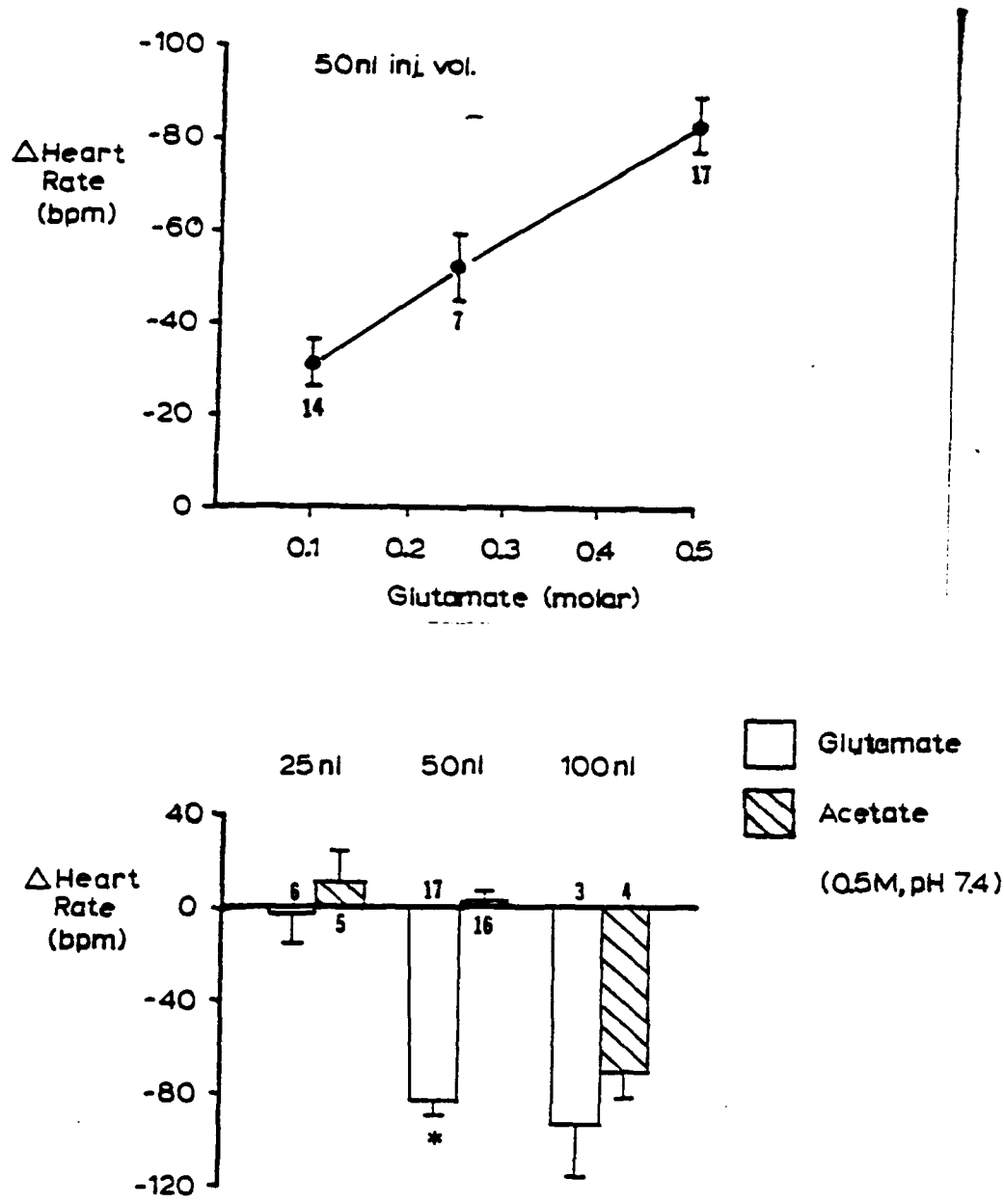

Figure 5. (top) The maximal change in heart rate to unilateral microinjection of varying concentrations of L-glutamate into the PVN. (bottom) The maximal change in heart rate to varying volumes of L-glutamate and acetate.

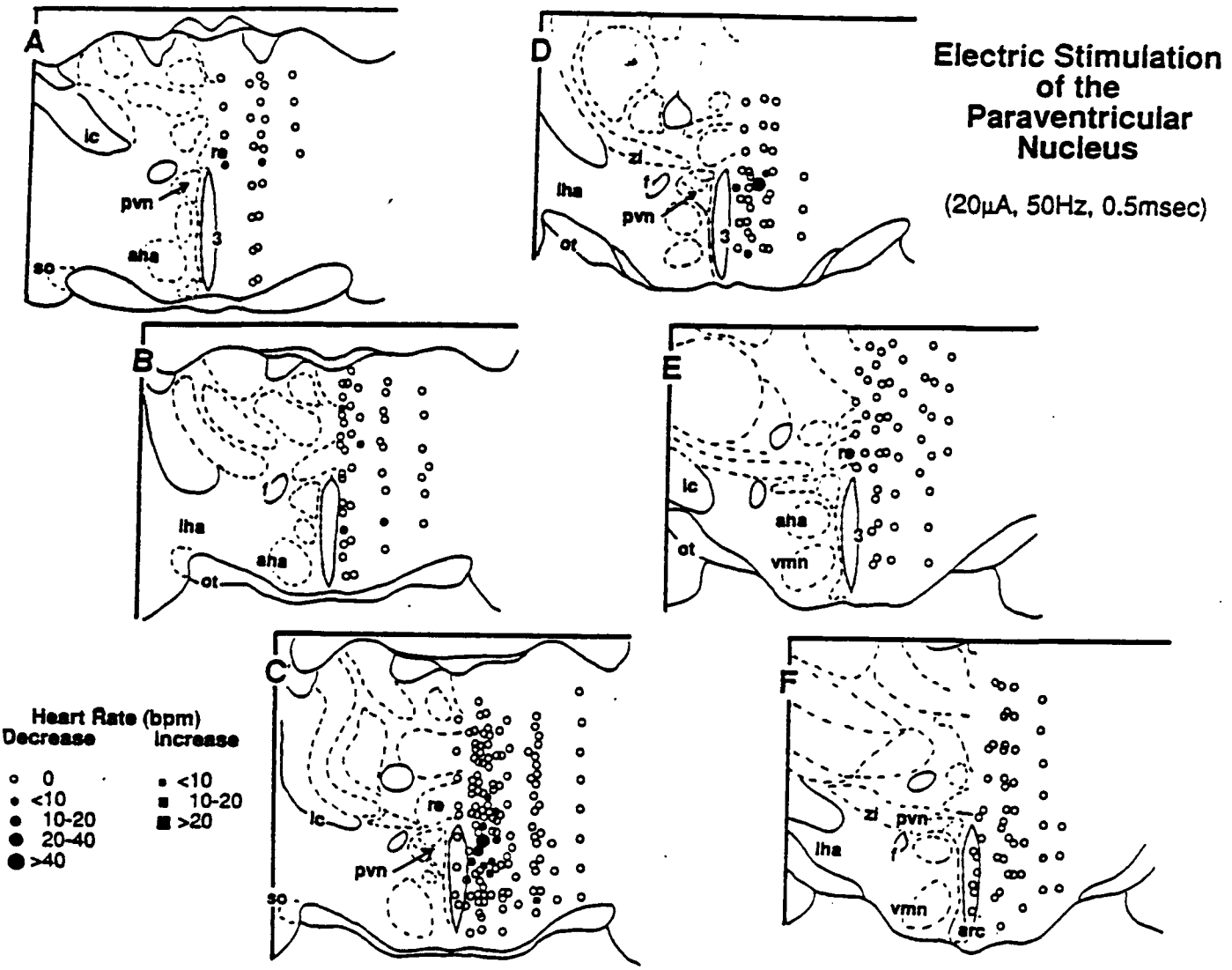

Figure 6. Focal electric stimulation of the PVN and regions around that area. Notice that bradycardia is elicited only when the PVN is stimulated. n = 14 rats, 2-4 stimulation tracks per rat. 7-8 stimulation sites per track.

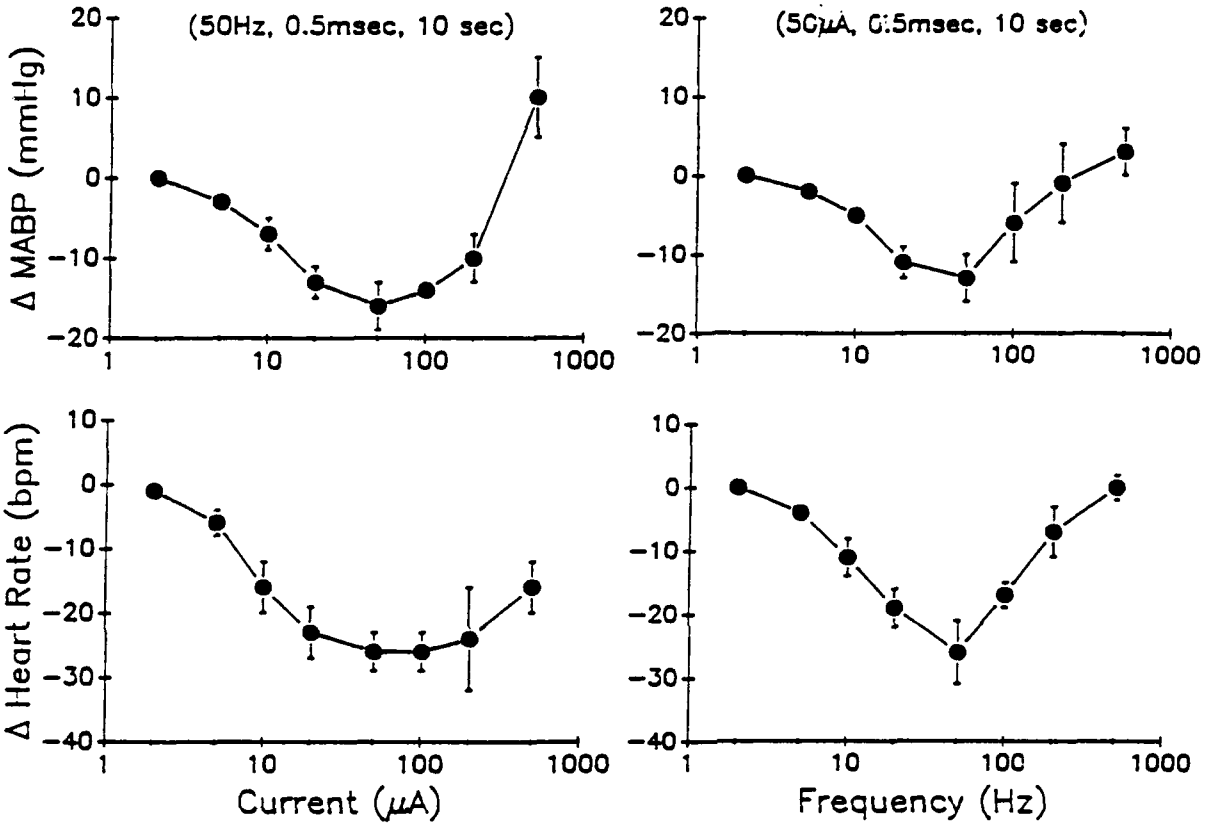

Figure 7 shows the maximal change in mean arterial blood pressure (MABP) and heart rate during electric stimulation of the PVN while varying current intensity (n=8) and frequency (n=7). Values represent mean±SE.

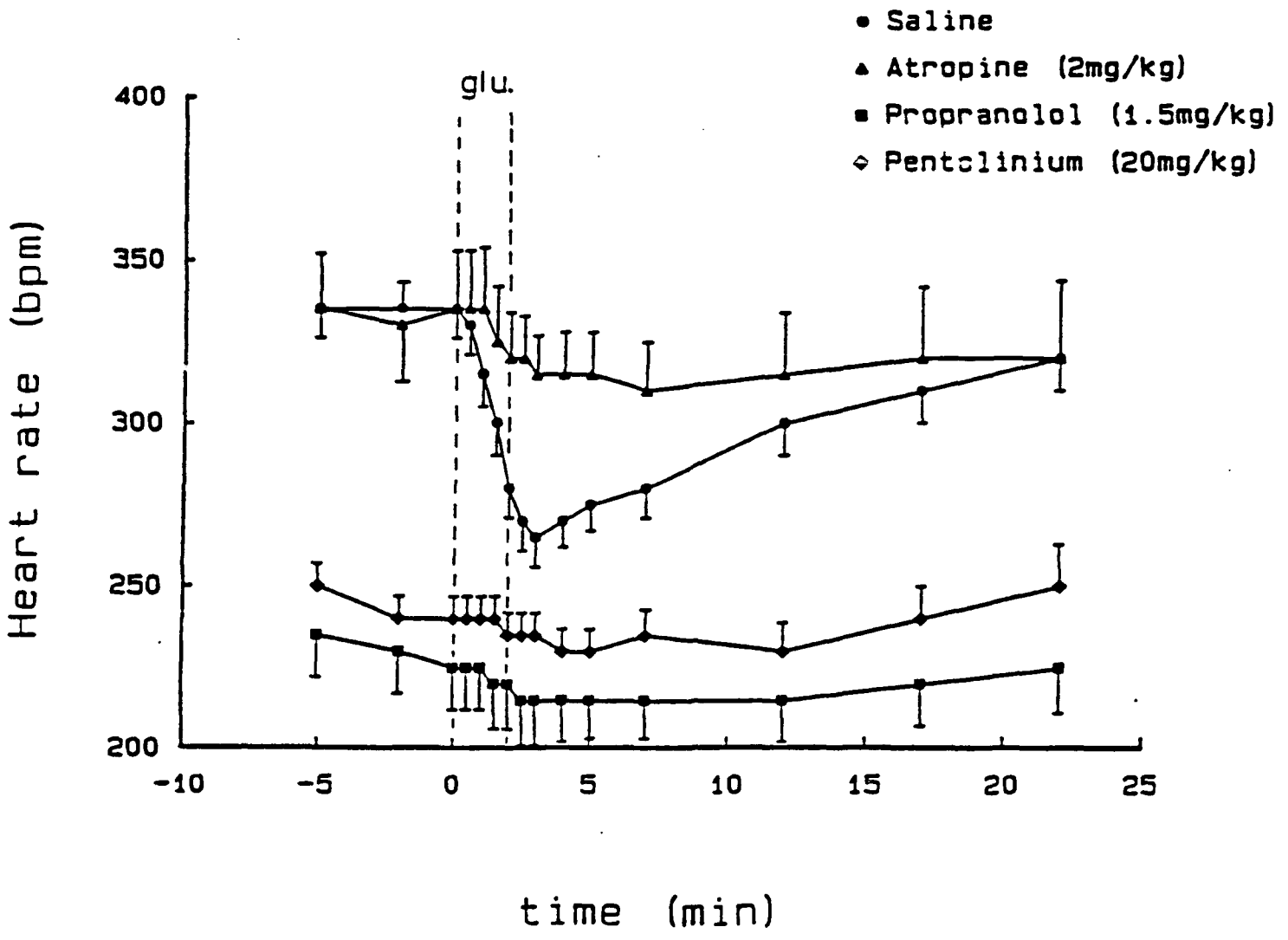

Figure 8. The effect of atropine, propranolol, pentolinium and vehicle (saline) pretreatment on the response of heart rate to unilateral microinjection of L-glutamate into the PVN. glu. - period of L-glutamate injection. Values represent mean $\pm$ SE. Saline- n = 26, atropine- n = 8, propranolol- n = 8, pentolinium- n = 10.

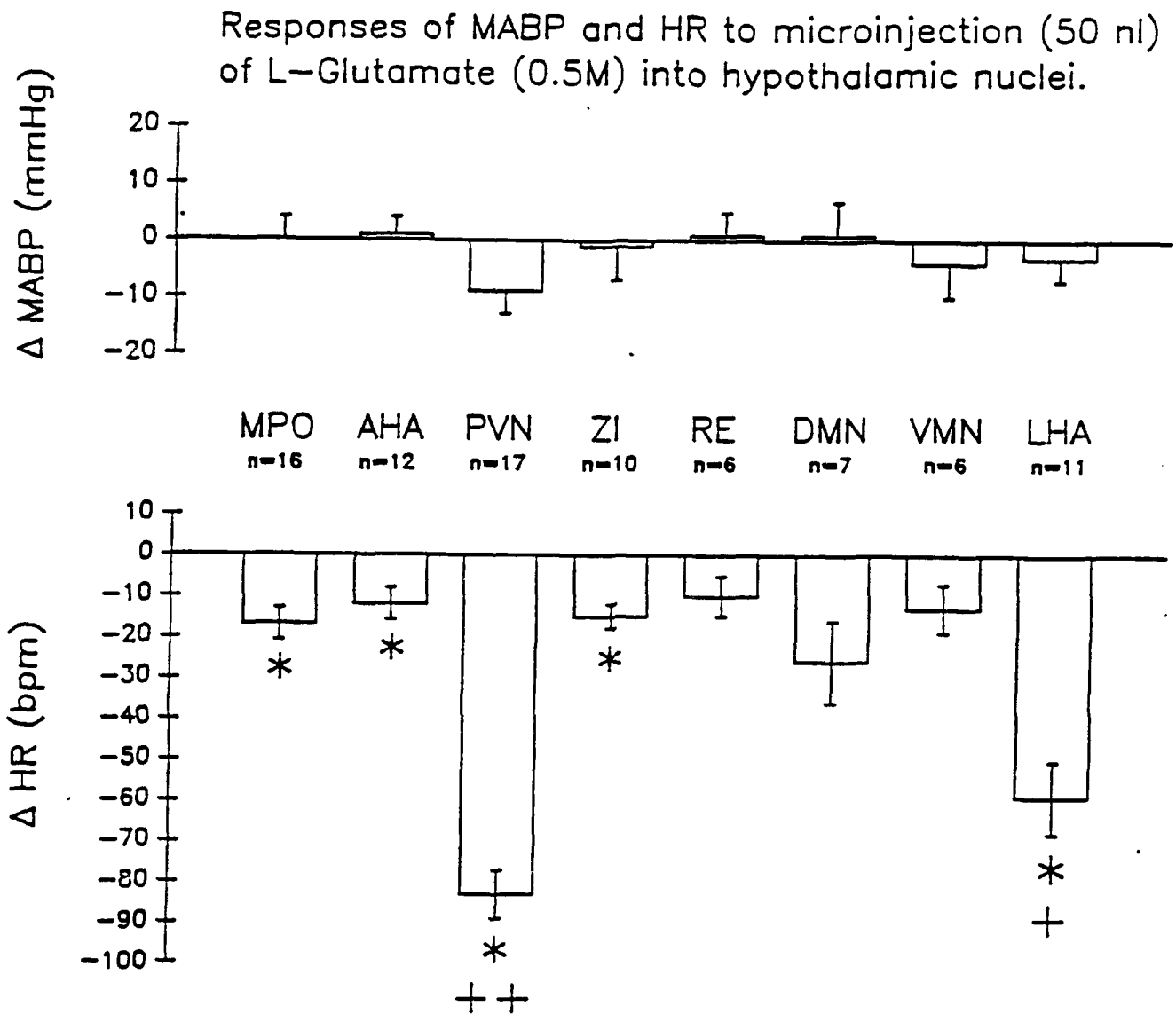

Figure 9. The maximal change in mean arterial blood pressure (MABP) and heart rate to activation of cell bodies in nuclei around the PVN. MPO-medial preoptic area, AHA-anterior hypothalamic area, PVN-paraventricular nucleus, ZI-zona incerta, RE-nucleus reuniens, DMN-dorsal medial nucleus, VMN-ventral medial nucleus, LHA-lateral hypothalamic area. Values represent mean $\pm$ SE. * represents a significant response; + LHA response significantly different from all other responses; + + PVN response significantly different from all other responses.

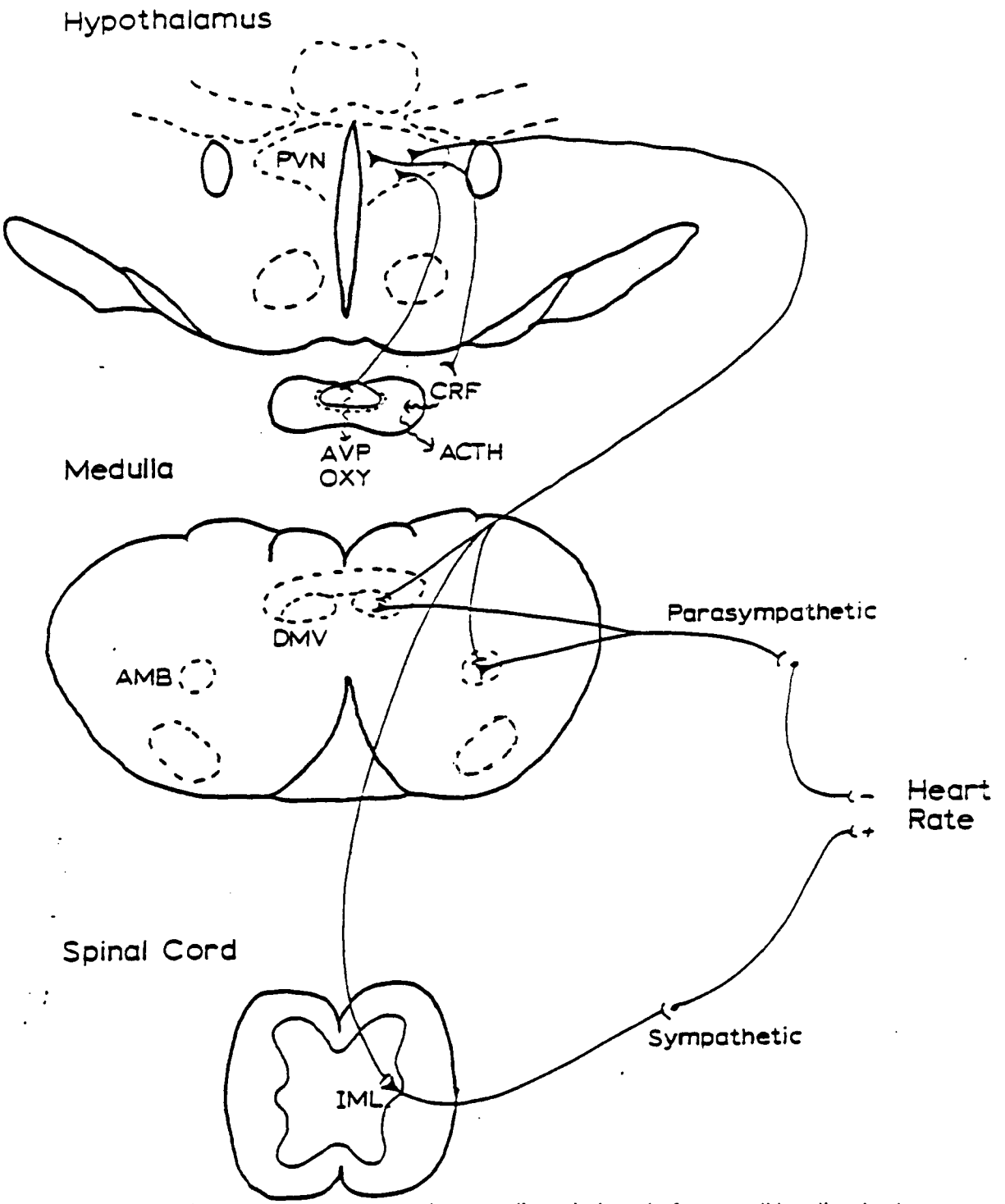

Figure 10. Neural pathways proposed to mediated signals from cell bodies in the Paraventricular nucleus (PVN) to the Dorsal Motor Nucleus of the Vagus (DMV), Nucleus Ambiquus (AMB) and the Intermediolateral Column (IML) of the Spinal Cord for control of heart rate.

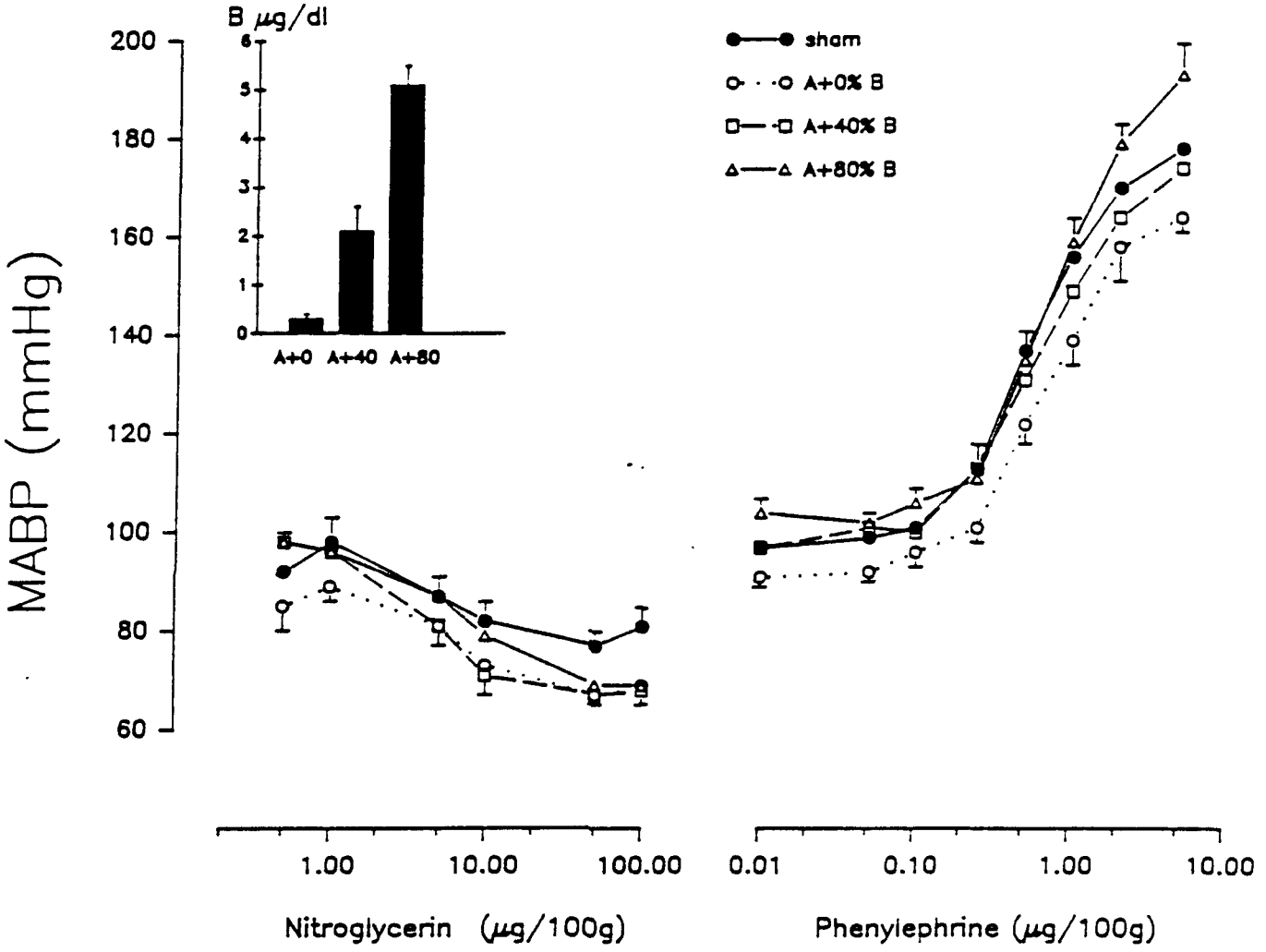

Figure 11. Mean arterial blood pressure (MABP) after intravenous injection of nitroglycerin or phenylephrine (per 100g of body weight). Inset in the upper left corner represents corticosterone (B) levels measured before experiment. Values represent mean±SE. $n = 7$-10 per group.

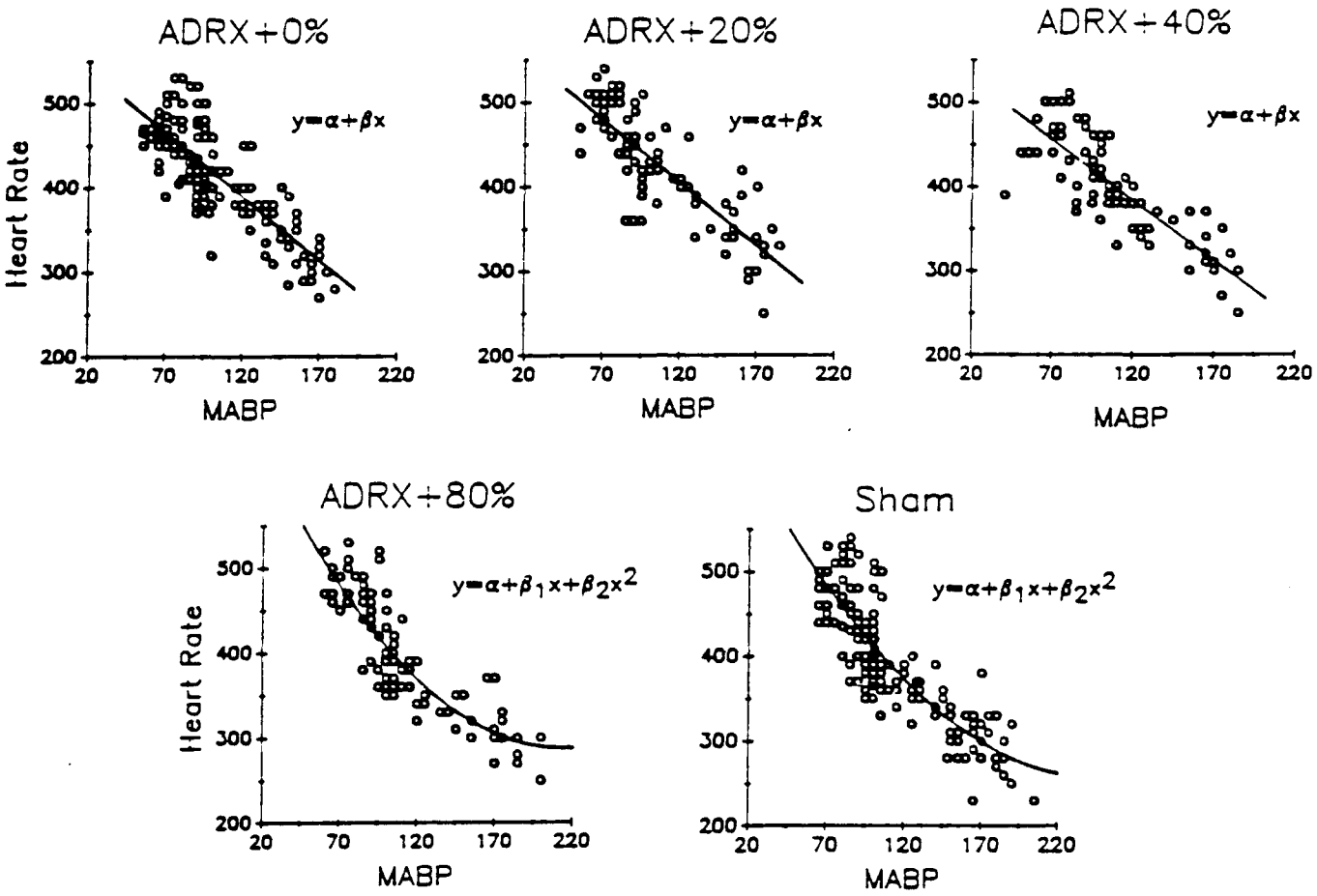

Figure 12. Baroreflex curves in conscious adrenalectomized (ADRX) rats without and with corticosterone replacement (pellets 0,20,40 and 80% mixed with cholesterol). Heart rate was recorded as mean arterial blood pressure was manipulated by bolus injections of varying doses of phenylephrine or nitroglycerin. Simple linear regressions best fit the ADRX + 0, 20 and 40% data. By contrast, quadratic functions best fit the data from Sham and ADRX + 80% groups; the coefficients were not different between Sham and ADRX + 80% groups, strongly suggesting the responses were the same.

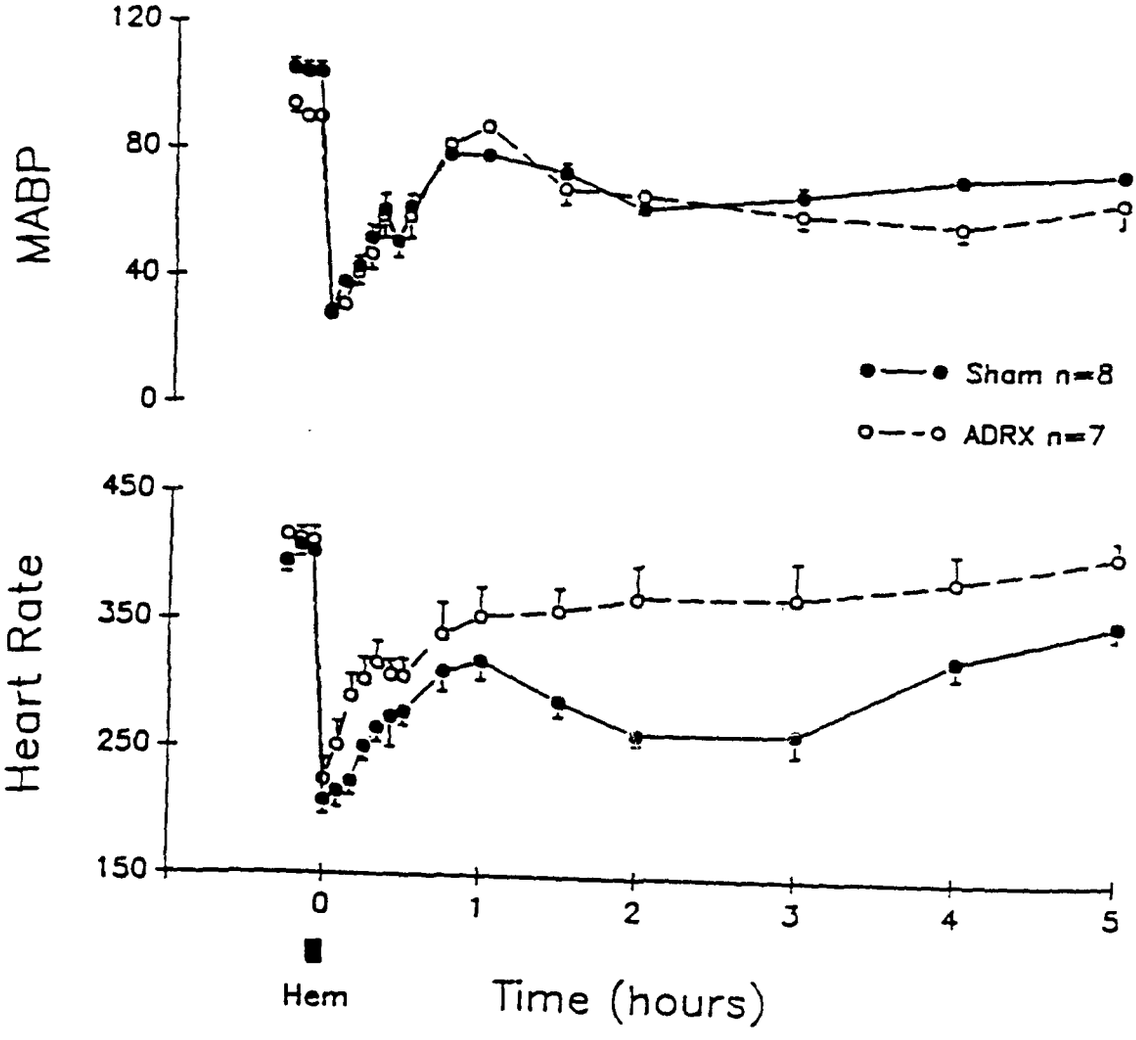

Figure 13. The responses of mean arterial blood pressure (MABP) and heart rate to 15ml/kg*5min hemorrhage in conscious adrenalectomized (ADRX) and sham-adrenalectomized rats.

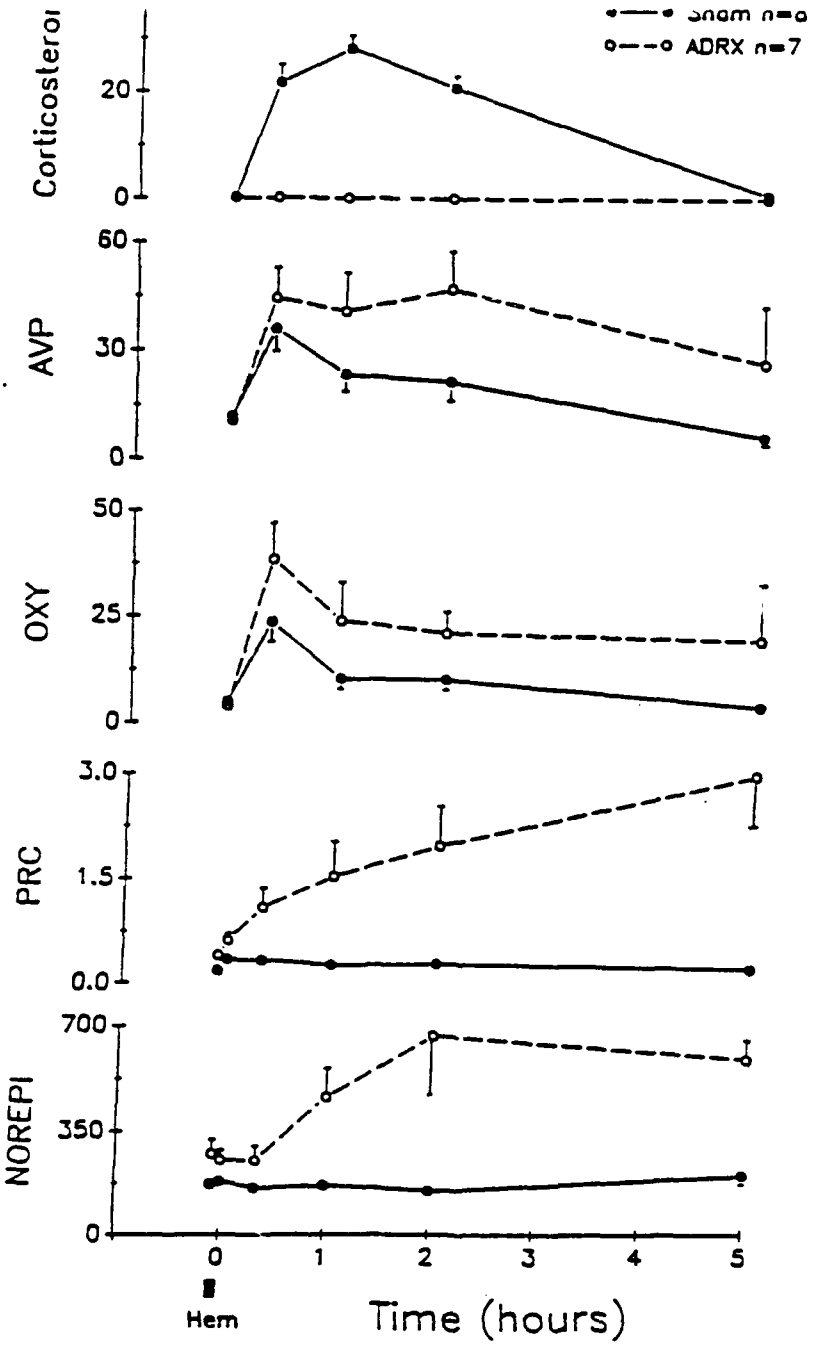

Figure 14. The responses of plasma corticosterone (ug/dl), vasopressin (AVP, pg/ml), oxytocin (OXY, pg/ml), plasma renin concentration (PRC, mg AII/ml), and norepinephrine (norepi, pg/ml) after 15ml/kg*5min in conscious ADRX and Sham rats.

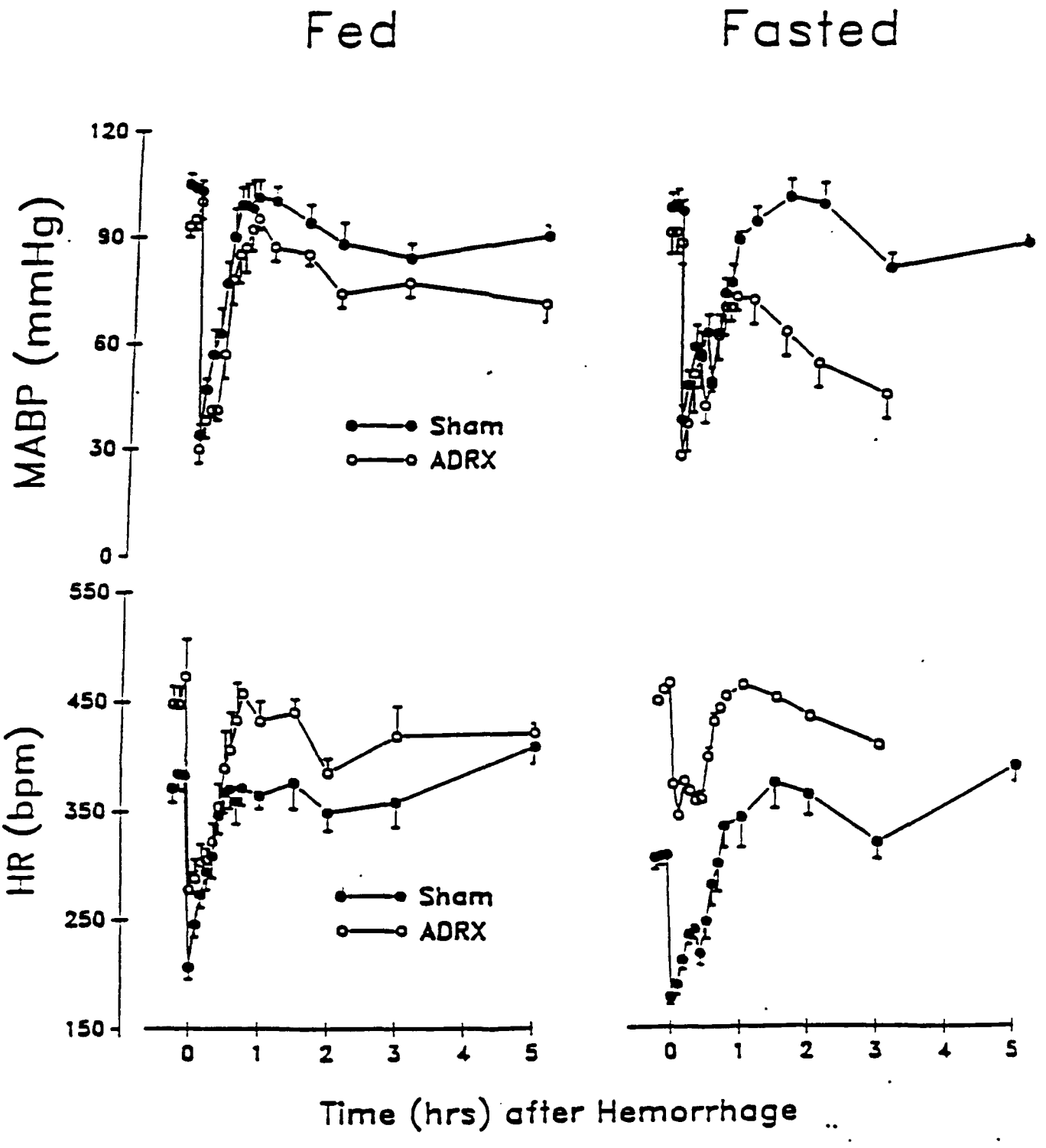

Figure 15. The responses of mean arterial blood pressure (MABP) and Heart Rate (HR) to 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX) rats. There was a significant difference (2-Way ANOVA) between the Sham and ADRX groups for the responses of MABP and HR (for both fed and fasted treatments). Values represent means $\pm$ SE.

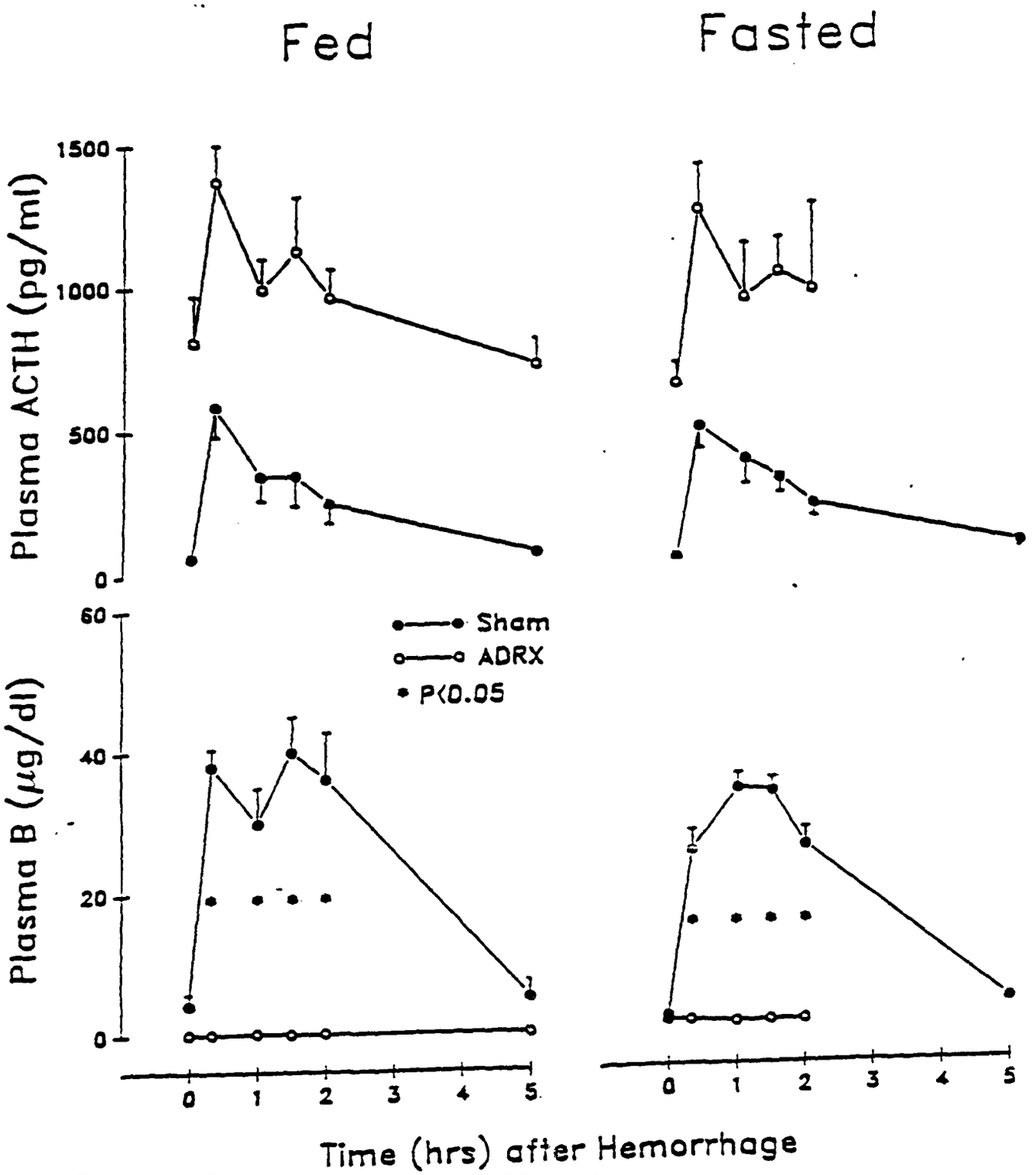

Figure 16. The responses of plasma ACTH and corticosterone after 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX). There was a significant difference (2-Way ANOVA) between the Sham and ADRX groups for the responses of ACTH and corticosterone (for both fed and fasted treatments). Values represent means $\pm$ SE.

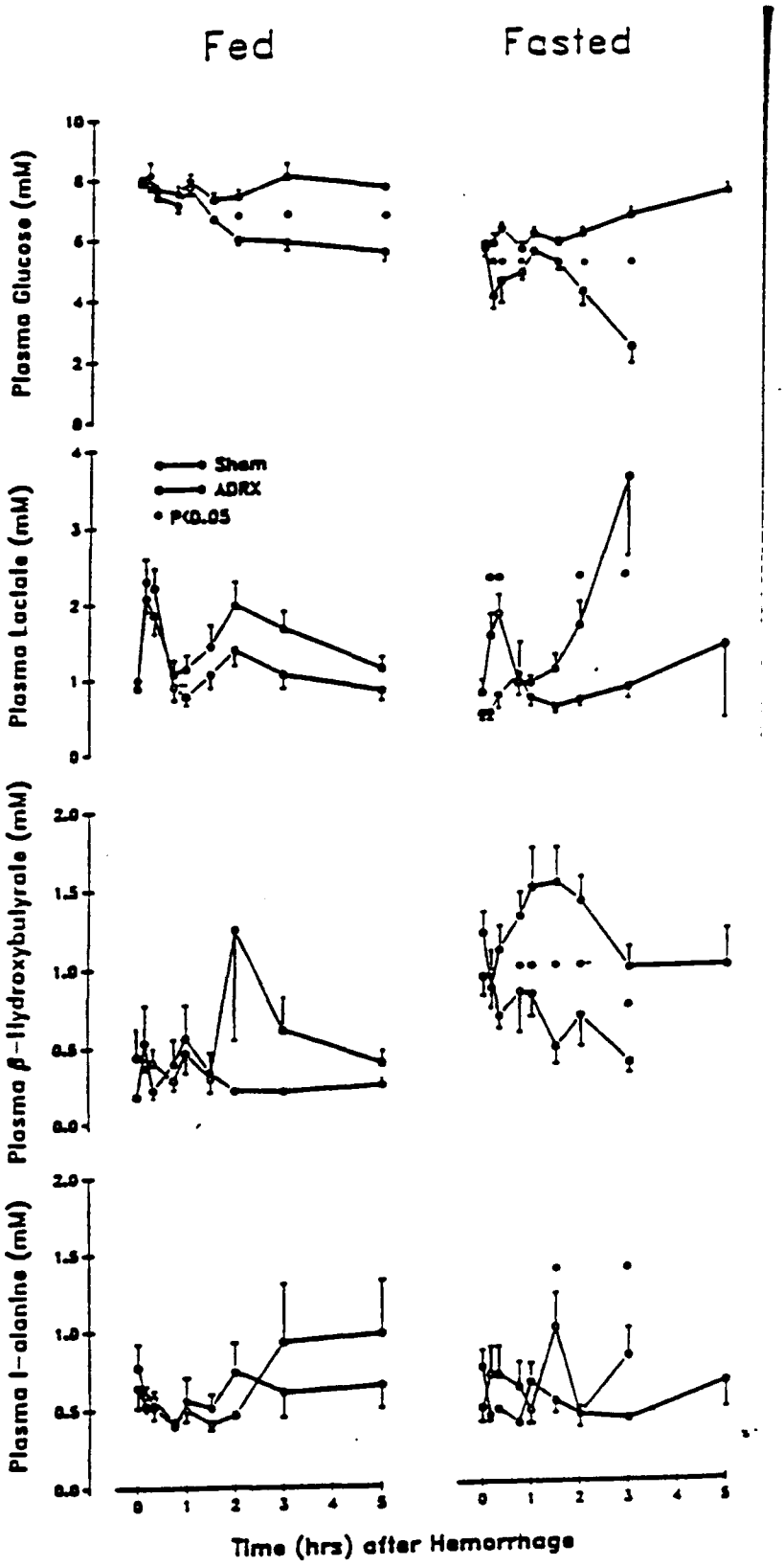

Figure 17. The responses of plasma glucose, lactate, B-hydroxybutyrate and l-alanine after 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX) rats. There was a significant difference (2-Way ANOVA) between the Fasted Sham and ADRX groups for the responses of all plasma substrates and between the fed Sham and ADRX groups for the responses of glucose. * Significant difference at that time point by Newman-Keuls. Values represent means $\pm$ SE.

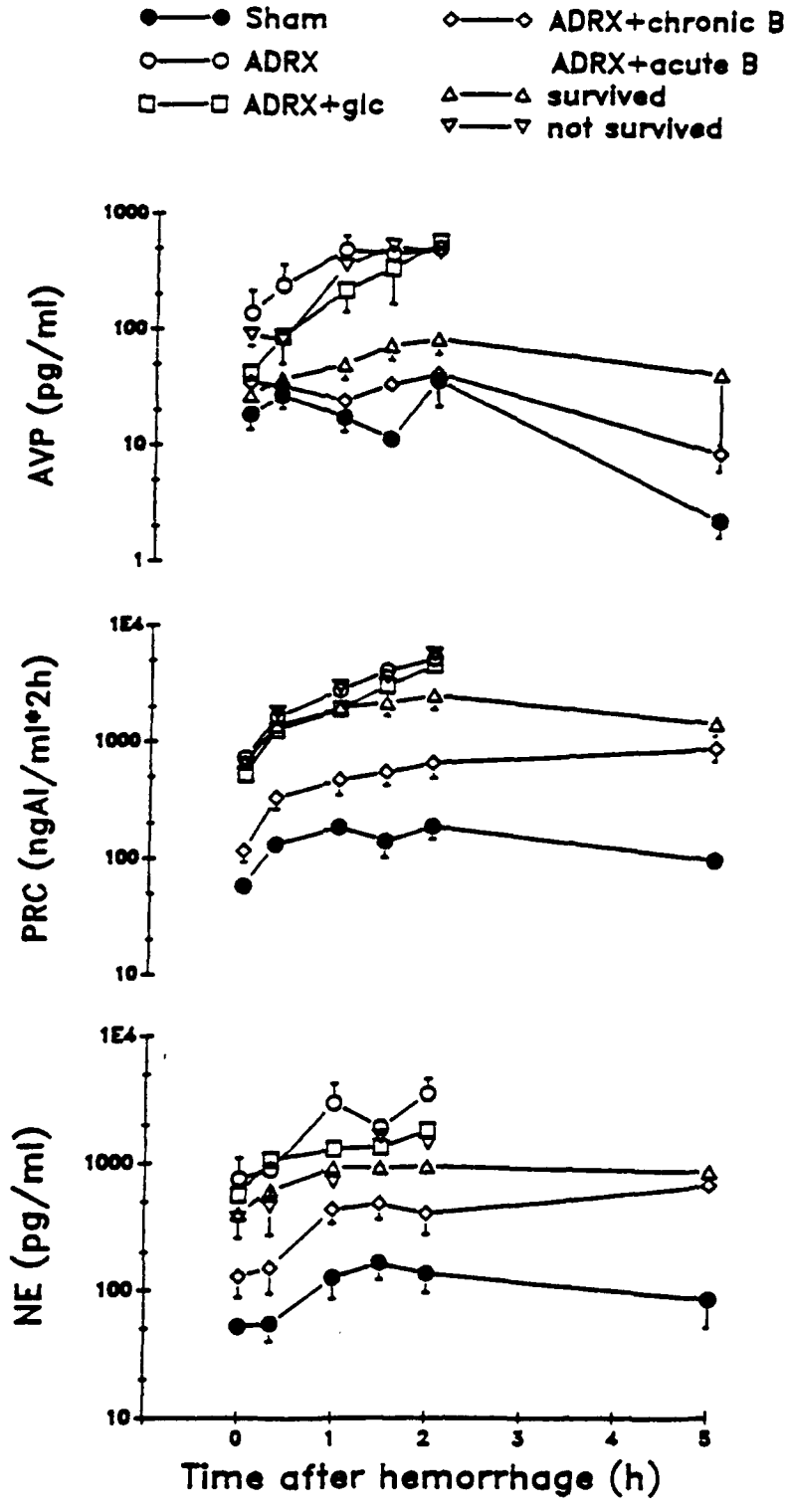

Figure 18. Response of plasma vasopressin (AVP), plasma renin concentration (PRC) and norepinephrine (NE) to 15ml/kg*5min hemorrhage in fasted sham and fasted adrenalectomized conscious rats given glucose (glc, 60mg/hr), a chronic corticosterone (B) pellet, and a acute infusion of B (70μg/hr).

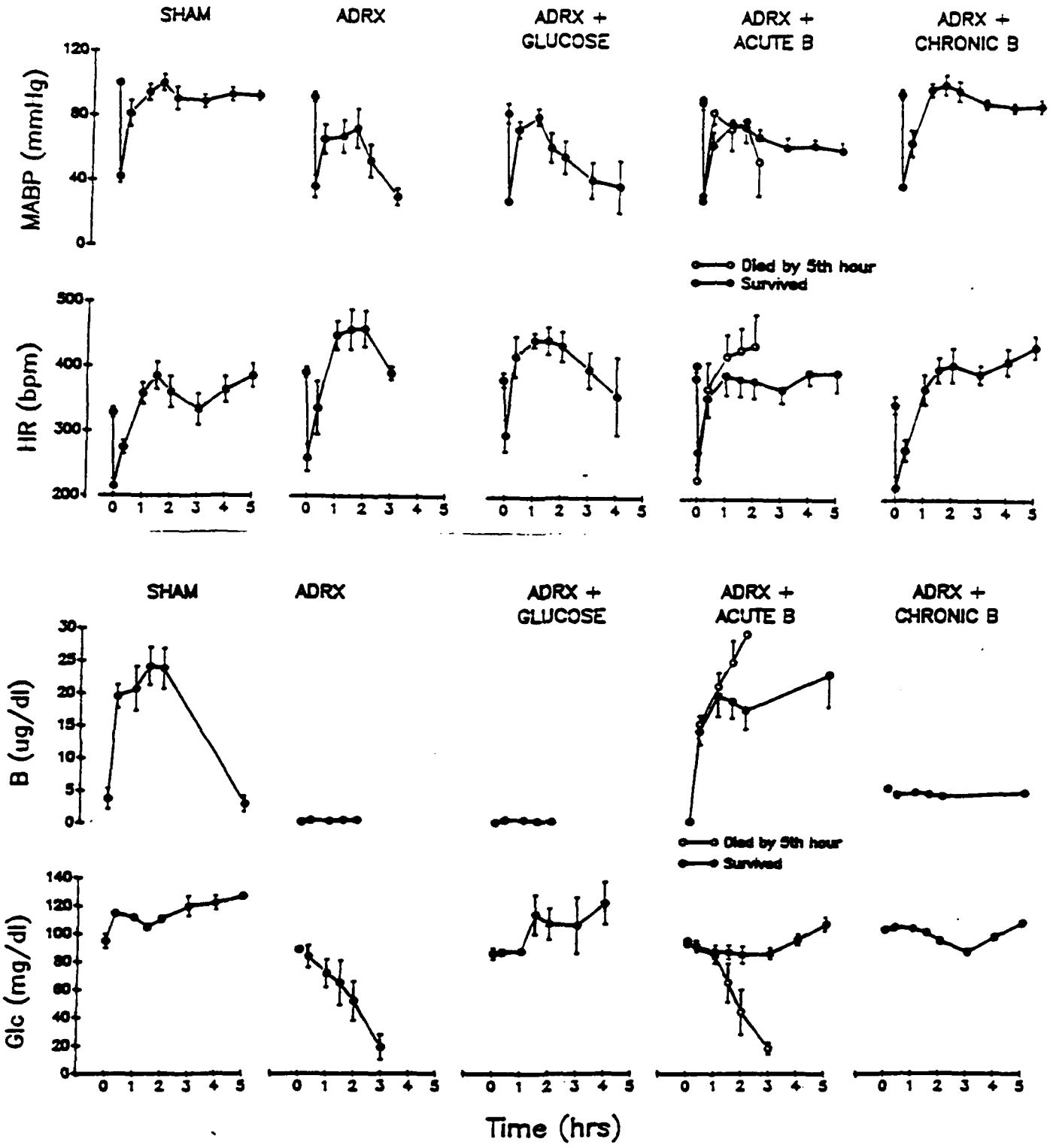

Figure 19. Mean arterial blood pressure (MABP), heart rate (HR), plasma corticosterone (B) and plasma glucose responses to 15ml/kg*5min hemorrhage completed at zero time in fasted sham and adrenalectomized rats given glucose (glc, 60mg/hr at 1hr), an acute infusion of B (70mg/hr at time 0) and a B pellet 4 days prior.

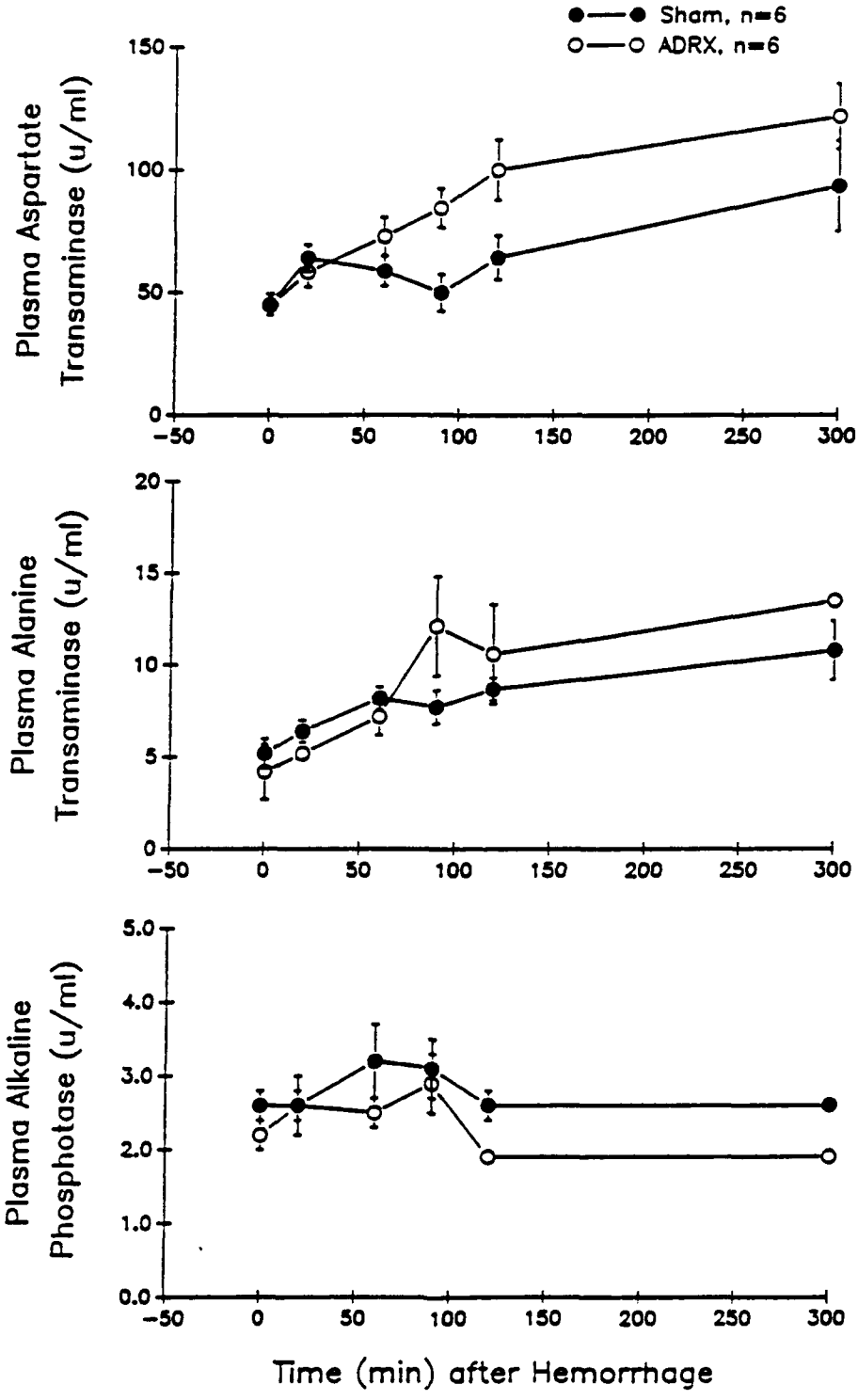

Figure 20. Plasma levels of aspartate and alanine transaminase and alkaline phosphatase (in Sigma units/ml) following 15ml/kg*3min hemorrhage in fasted sham and adrenalectomized rats.